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EP-A- 0 240 191
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PHARMACIA, FPLC SYSTEM, HIGH PERFORMANCE PURIFICATION OF BIOMOLECULES, September 1986, Rahms i Lund, Uppsala (SE); pp. 2-46—

PHARMACIA, FPLC: MEDIA AND COLUMN GUIDE, HIGH PERFORMANCE SEPARATION OF BIOMOLECULES, Uppsala (SE); pp. 1-16—

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PATENT ABSTRACTS OF JAPAN, vol. 11, no.
13 (C-397)[2460], 14 January 1987&NUM;

J. CHROMATOGR. LIB., vol. 31, 1985; pp.
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J. CHROMATOGR. LIB., vol. 41(b), 1988; pp.
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SCIENCE, vol. 222, 1983; pp. 245-252&NUM;

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1174-1183&NUM;

J. CHROMATOGR., vol. 266, 1983; pp.
385-394&NUM;

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339-353&NUM;

J. CHROMATOGR., vol. 107, 1975; pp.
125-140&NUM;

Description

The present invention relates to the separation of long-chain nucleic acids from other substances from solutions containing nucleic acids and other materials, and more particularly nucleic acid/protein mixtures from biotechnical preparations from bacteria, viruses, animal and vegetable tissues and cells, more particularly cell ingredients and/or degradation products thereof as well as components of body liquids which components are not long-chain nucleic acids, and to the device for carrying out the method.

5 The preparation of nucleic acid from natural sources, and more particularly from viruses, bacterial and eucaryotic cells, cell aggregates or tissues as well as body liquids is a key technique for various preparative 10 and analytical problem solutions in biology and medicine. Some important applications may be mentioned by way of example hereinafter:

Molecular biology uses vehicles capable of replicating for DNA fragments which include plasmides, 15 phages, viruses etc.. In order to be able to use the DNA- or RNA-processing enzymes, first a highly purified DNA or RNA is needed. The same is applicable to genetical analytics of, for example, viruses from tissue liquid or genomic DNA from tissue. Since for a specific detection of certain characteristics of nucleic acids such as, for example, restriction polymorphisms, said nucleic acids prior to analysis are subjected to an enzymatic degradation, they must be present in such a purity that these methods of enzymatic degradation are usable. The methods so far known do not allow to extract and to concentrate the DNA/RNA by following similar and simple instructions for operation from starting materials being so different as solutions 20 containing nucleic acids and other materials, more particularly nucleic acid/protein mixtures from a biotechnological preparation, tissue, blood, sputum, cell cultures, bacteria, fungi, renal and fecal excrements.

The problems will become more clearly evident in consideration of virus diagnostics, for example the detection of Hepatitis B-DNA in blood and liver biopsies, the individual assignment in criminalistics, forensic 25 medicine or paternity analysis, wherein the analytical methods to be employed require cellular nucleic acids from very different sources such as sperms, tissue (fresh, carbonized, frozen, dried etc.) for use in the technically comparable kind of subsequent analysis.

The methods as so far known for the purification of long-chain nucleic acids require centrifugation steps of extended duration or aqueous phenol/two-phase extractions. Such procedures are rather intensive in 30 personnel and equipment cost and, moreover, too expensive to be simply realizable in an automated operation. Furthermore, the known and conventionally used purification methods involve the use of expensive equipment such as cooled centrifuges and ultracentrifuges which, in addition, consume valuable materials such as cesium chloride for density gradient centrifugation and rotor insertions for one-time use.

A method described in EP-A- 0 104 210 and based on the use of HPLC devices is suitable for a 35 chromatographic separation of nucleic acids, however, long-chain nucleic acids such as, for example, λ -phage DNA, are damaged by the mechanical action.

The Journal of Chromatography Library, vol. 31 discloses in "gradient elution in column liquid chromatography, theory and practice" P. Jandera and J. Churacek (p. 383), that chromatography on hydroxylapatite and polyamino acid-diatomaceous earth columns is the best method for separating DNA 40 from RNA and higher oligonucleotides for removing proteins and for separating native and denatured DNA. Chromatography on anion-exchange columns results, however, in fractionation into many peaks, usually not all of which are fully characterized. Stepwise or continuous gradients of sodium chloride in a suitable buffer are used for elution. A gradient of urea can also be used. It is also mentioned that anion exchangers chemically bonded on a silica gel support, however, with a particle size of 10 μm have been tested giving a 45 speed up analysis.

Kato et al. describe in Journal of Chromatography, 266 (1983) 385 - 394 disclose high-performance ion-exchange chromatography of proteins with silica-based column materials of pore diameter 250 - 300 Å which are also suitable for separation of nucleic acid. The particle size of the column material disclosed is 10 μm . This means that only high-pressure liquid chromatography applications are disclosed. As nucleic 50 acids there are separated tRNA, rRNA of different sizes as well as DNA fragments of various sizes up to 590 base pairs were used.

Riesner et al., Journal of Chromatography, 296, 339 - 353 (1984) disclose the separation of nucleic acids with high-pressure liquid chromatography by using a material having a particle size of 10 μm . Also this reference describes the elution of nucleic acids via a continuous salt gradient.

55 Millard et al., Journal of Chromatography, 107 (1975), pages 125 - 140 disclose the chromatography of genomic DEAE-cellulose. DEAE-cellulose belongs to the non-porous chromatographic materials.

Cherayil et al. in Biochemistry, 1174 to 1183 (1965) there is disclosed separation of sRNA on DEAE-cellulose and DEAE-Sephadex. These nucleic acids have globular structures. These materials are not able

to separate long-chain nucleic acids which have a more rigid rod-like structure as for examples plasmids, lambda- or M13-phages and the like.

A method for isolation and purification of plasmid DNA is described in patent abstracts of Japan, vol. 11, number 13 (C-397) [2460]. A cyclic double-stranded DNA containing RNA and other DNA is subjected

- 5 to a specific gel filtration chromatography to effect short-time and economical isolation and purification of plasmid DNA. A cyclic double-stranded DNA containing other components is previously treated with ribonuclease to digest a part of RNA, then subjected to gel filtration chromatography of spherical, hydrophilic semirigid vinyl polymer gel which has hydroxyl groups and ether groups on its side chains and swells in aqueous solutions, more than 1000 Å average pore diameter and less than 100 µm average
- 10 particle size using as an eluate, an aqueous solution of 7.0 - 8.5 pH containing 2 - 500 mM of TRIS hydrochloride, 1 - 50 mM of EDTA and 10 - 1000 mM of NaCl to separate and purify a plasmid DNA of 3 - 12 Kbp.

EP-A-0 240 191 describes a method of isolating DNA contained in a virus or a cell comprises the steps of; isolating the virus or cell having satellite DNA; reacting a protein decomposer with said virus or cell for destroying or denaturing protein covering DNA; adding at least twice by volume of alcohol to the reaction mixture for precipitating DNA; feeding an alcohol containing solution obtained in the previous step into a separation column for retaining the precipitated DNA; passing an aqueous alcohol solution having a high alcohol concentration through the column for removing impurity; and passing an aqueous solution having a low alcohol concentration or an aqueous solution free from alcohol through said column for eluting said DNA.

From Bernardi, G. (1971), "Methods in Enzymology" (Grossman, L. & Moldave, K., Edit.) Vol. 21, pages 95 to 139, Academic Press, New York, there has been known a method for the separation of nucleic acids from proteins, lower molecular weight substances and cellular components such as oligo- and polysaccharides by chromatographic purification on hydroxylapatite (HAP). This method has also been used for the purification of plasmides and λ-phage DNA (cf. Colman, A. et al, 1978, Eur. J. Biochem. 91, 303 to 310; Shoyab, M. & Sen, A., 1978, J. Biol. Chem. 253, 6654 to 6656; and Johnson, T.R. & Ilan, J., 1983, Anal. Biochem. 132, 20 to 25). However, this method is not comparable to the method according to the invention. Thus, for example, the separation efficiency, expressed in milligrams of nucleic acid per grams of separating gel, which amounts to about 1 mg/1 g in the method according to the invention is about 100 times higher than that of the HAP method. For long-chain nucleic acids the separation on HAP results in high losses in yield, more specifically of cellular DNA, and requires high phosphate and urea concentrations in the eluting buffer, which adversely affects further processing of the separated long-chain DNA. The known gel permeation procedures are not capable of separating high molecular weight nucleic acids from other high molecular weight substances such as proteins and polysaccharides, since these materials will only select by size and shape.

For the direct hybridization reaction the product purity as obtained by known methods is usually sufficient. However, for a number of detection problems the concentration of the purified long-chain nucleic acid is too low for allowing direct detection by hybridization.

As examples there may be mentioned the analysis of AIDS virus nucleic acids in much underrepresented infected cells of a lymph node biopsy or the detection of a restriction fragment length polymorphism (RFLP) in a small amount of cells obtained upon an amniocentesis or chorion biopsy.

If specific nucleic acid sequences are to be enzymatically amplified, then the nucleic acid to be amplified must be present in such a purity that enzymes such as polymerases will not be inhibited (Saiki, R.K. et al., 1985, Science 230, 1350 to 1354). An essential purification step of the known methods is the use of a phenolic extraction in order to efficiently effect the removal of proteins and organic agents which may inhibit enzymes. However, phenol is a strong poison to skin and liver and should be processed only by well trained staff under strict precautions. Moreover, liquid extractions are time-consuming and intensive in personnel.

So far such purifications of long-chain nucleic acids, more particularly in molecular biology, could be carried out only in research institutes, for the known methods are time-consuming and intensive in instrumentation and cost and, moreover, due to the used chemicals is dangerous to health. A typical instruction for operation may be classified into the following steps:

- 55 a) the disintegration and digestion of the cells or tissues or body liquids for which a number of methods may be employed such as mechanical methods (for example milling) in combination with other physical methods (for example a boiling procedure - "Koch-Verfahren"), with enzymatic methods (using, for example, proteinase K, lysozyme etc.) and with chemical methods (using, for example, sodium hydroxide solution, diethyl pyrocarbonate) and which renders the cell contents accessible to further enzymes and reagents;

- b) a coarse clarification of the solution from cell debris by means of a centrifuge;
- c) steps for the removal of proteins and first accumulation of the nucleic acid, usually by utilization of a two-phase system consisting of phenolic phase/aqueous phase; and
- d) high purification techniques such as ultracentrifugation.

5 The known methods for purifying long-chain nucleic acids ($> 20 \text{ kB} \approx \text{molecular weight} > 13 \text{ million Dalton}$) have in common that they are difficult to rationalize if the nucleic acid preparations are to be carried out as routine operations. Such condition, for example, exists in laboratories of molecular biology which permanently have to provide highly pure plasmides or phage DNA.

10 In medical diagnostics there is an urgent demand to obtain new information and knowledge from the analysis of genetic material. Hereto, the problem of hepatitis diagnostics may be mentioned, where only the direct detection of the virus will provide information on the infectiosity, or the genetic detection of a genetically caused protein deficiency, for example of a thalassemia. The work-up of the material to be analyzed (DNA or RNA), more particularly with large sample numbers, has proven to be crucial barrier on the route to a genetics-based diagnostics, if the latter should match the known serologic methods with respect to the applicability thereof to large sample populations.

15 The importance of an automated nucleic acid work-up is extremely high. This mode of operation is a pre-requisite for a generally applicable genetics-based diagnostics which with respect to the importance thereof could correspond to the widely used methods of serologic diagnostics. Both methods cover areas, the respective information obtainable from which will add up to each other in a complementary manner.

20 While in immunology the cell or virus products could be qualitatively and quantitatively determined, by genomic analysis the diagnosis is verified on the level of the information store of the nucleic acid.

25 Gene technology enables an extremely high-resolving diagnostics to be effected due to the fact that nearly each individual structural element of the genetic store comprising up to billions of structural elements can be examined. The procedure allows to determine the presence or absence of infectious genetic material, for example of an AIDS causationist, or to recognize genetic diseases such as muscle dystrophia without gene products having to be expressed, for example in the form of protein/antigens, or the absence thereof having to be determined.

30 Furthermore, biotechnology, and more specifically gene technology, enables products to be produced by means of transformed microorganisms. However, under this aspect there arises a very serious problem from that it cannot be excluded that upon use of products having been prepared by biotechnology potentially noxious genetic information is taken over into the cell or into the genetic information, respectively, of the user and there cause transformation, infection, resistance to antibiotics etc. to occur.

35 The problem is all the more serious as recently increasingly homologous transformed cell systems are employed in the place of organisms such as *E. coli*. or yeast, such as, e.g., hamster ovar cells, human fibroblasts, cancer cells etc.. This is pursuant to the goal, if possible, to produce human-identical protein products which with respect to conformation and, above all, modification such as glycosylation and other post-translational modifications are equal to human proteins.

40 However, simultaneously therewith the danger is enhanced of a possible transformation of human cells of a patient by homologous DNA sequences or adapted vector systems having their properties such as self-reproducibility, resistance behavior, presence of strong promotors, enhancer elements, oncogenetic information such as "gene dose" effects. Such apprehensions were uttered also with respect to genetic information obtained from *E. coli*, yeast, *B. subtilis* etc.. Thus, the danger exists of that preparations contain nucleic acid which either directly acts as a pathogen, such as in the case of certain viruses and of oncogenetic DNA, or which may indirectly act to initiate a cancer by becoming integrated in the receptor DNA and initiating mutations thereupon. This is why it is desirable that all therapeutic products as much as possible are free from nucleic acids. Thus, the American health authorities {Food and Drug Administration (FDA)} for the time being recommends that not more than a dose of 1 to 10 pg/day of DNA should be administered.

45 Upon application of the methods according to prior art, depending on the kind of producing system, in the first steps of purification of the biotechnically prepared products varying amounts of nucleic acid are obtained. Only traces of contaminating nucleic acid are present once the cells continuously secrete the synthetized product so that only undesiredly lysed cells will significantly release nucleic acids. However, the total cell equivalent of nucleic acids may also occur as contamination, if after batch production the host cells are completely lysed for intended product release or being killed. In this latter case the first step frequently is the precipitation of the DNA/RNA by polycations such as polyimine. However, this step does not lead to a complete removal of the substances.

50 Therefore, it is the object of the present invention to provide a process for removing long-chain nucleic acids from tissues and body liquids which

- a) in a similar manner allows the nucleic acids to be extracted and concentrated from the most various starting materials such as tissue, blood, sputum, cell cultures, bacteria, fungi, renal and fecal excrements as well as vegetable tissue from callus cultures, roots etc.,
- 5 b) requires no long-time centrifugation steps, and more specifically no ultracentrifugation,
- c) can be carried out without expensive equipment, and more specifically without refrigerated centrifuges and ultracentrifuges, and without using valuable material such as cesium chloride for density gradients or rotor insertions for one-time use,
- d) ensures high purity of the nucleic acid to be attained,
- e) works without phenolic extraction step, and
- 10 f) is suitable for being automated,

and by means of extraction of the long-chain nucleic acid separates mixtures of long-chain nucleic acids and other materials such as those obtained when products are biotechnologically produced.

In the EP-A- 0 104 210 there has been described a method for separating nucleic acids up to plasmide size ($< 10\ 000$ base pairs ≈ 6 million Dalton). By using the material described therein which is distinguished by that a highly porous silicagel provided with an anion exchanger coating and employed in HPLC chromatography is used as a carrier, for example, pre-purified plasmides may be prepared in a highly pure state. Nevertheless, here also centrifugation steps and precipitation steps are necessary which are not suitable for application in bulk analysis and preparation, respectively. One crucial drawback consists of that for larger molecules, for example λ -phage DNA, during the chromatographic separation of particles $< 20\ \mu\text{m}$ the shear forces become so high that intact molecules cannot be recovered any more. This is all the more applicable to cellular DNA having the multiple length of λ -phage DNA.

The object of the present invention is attained by a method for the separation of long-chain nucleic acids from other substances from solutions containing nucleic acids and other materials avoiding the step of a chloroform and/or phenol extraction and/or density gradient centrifugation wherein the long-chain nucleic acids are fixed on a porous anion exchanger the anion exchanger having a particle size of from 15 to 250 μm and a pore diameter of 50 to 2500 nm, whereas the substances to be separated therefrom are washed out from the anion exchanger using a washing solution having an ionic strength below the elution point of the long-chain nucleic acid to be separated,

30 and the fixed long-chain nucleic acids are subsequently removed from the anion exchanger using a washing solution of high ionic strength.

The porous anion exchanger preferably consists of a material for chromatography on the base of silicagel, diatomite, aluminum oxide, titanium dioxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the above-mentioned carrier materials, the surfaces of which have been modified with chemical groups exhibiting anion exchanger activities.

In a preferred embodiment the porous matrix consists of modified silicagel particles having a particle size of from 15 to 250 μm , and preferably from 25 to 40 μm . The pores have diameters of from 100 to 2,500 nm, and preferably of about 400 nm. The modification of the silicagel is preferred to be effected by reacting the carrier material to form a silanating reagent to form an anion exchanger.

As has been disclosed in the EP-A- 0 104 210 this reaction employs γ -glycidyloxypropyl trimethoxysilane and N,N-dimethylaminoethanol as reactants.

The process according to the invention, *inter alia*, makes it possible to avoid a phenolic extraction of the digestion mixture for purifying the long-chain nucleic acids from interfering components.

45 In the process according to the invention must be used hydrophilic surfaces, since nucleic acids, and more particularly long-chain nucleic acids, tend to strongly interact with the matrix, if salt solutions of high ionic strength are used. The strong hydrophobic interactions may give rise to contamination and yield problems.

The mild enzymatic proteolysis may be carried out either alone or in combination with the application of mechanical means. A number of methods are available, namely mechanical methods (for example milling) in combination with other physical methods (for example a boiling procedure - "Koch-Verfahren"), enzymatic methods (using, for example, proteinase K, lysozyme etc.) and chemical methods (using, for example, sodium hydroxide solution, diethyl pyrocarbonate).

These methods may be employed either alone by themselves or in combination with the method according to the invention for the extraction of long-chain nucleic acids. Some of these known methods (T. Maniatis, E.F. Fritsch, J. Sambrook (CSH), 1982, "Molecular cloning" (C.S.H.)) utilize sodium dodecylsulfate (SDS) or Sarcosyl, as detergent or solubilizing and protein-denaturing agent. In the presence of more than 0.1% of SDS (preferred to be used are from 0.1 to 2%) the bond of DNA/RNA to the polycationic surface of

the carrier is affected and greatly reduced. If a use of SDS is inevitable for the digestion, then the aqueous phase must be admixed with phenol and/or chloroform, i.e. a liquid-liquid extraction is necessary in order to remove the SDS. An alternative is constituted by a step of solid phase extraction by means of hydrophobically coated carriers (reversed-phase carriers) prior to employing method according to the

5 invention.

In the method according to the invention the substances to be separated from the long-chain nucleic acids are removed by thoroughly washing them out with a washing solution of low ionic strength. The eluate formed is virtually free of long-chain nucleic acids. This is particularly advantageous in the removal of long-chain nucleic acids from products having been biotechnologically produced. The method according to the

10 invention allows a separation to be effected of more than 99% up to 100% of long-chain nucleic acids from nucleic acid/protein mixtures.

The porous anion exchanger employed in practising the method according to the present invention specifically complies with the following criteria which make it particularly useful for removing long-chain nucleic acids from nucleic acid/protein mixtures:

- 15 1. High affinity to long-chain nucleic acids;
- 2. low affinity to other materials, and more particularly to proteins;
- 3. no unspecific interactions with other materials such as proteins;
- 4. no unspecific retention of other materials, more specifically of proteins due to inclusions as physically caused (narrow pores);
- 20 5. sterilizability;
- 6. low bleed-off of the porous matrix;
- 7. no toxic decomposition products of the porous matrix;
- 8. high capacity of the porous matrix for nucleic acids;
- 9. regenerability;
- 25 10. physiological elution conditions; and
- 11. high process flow velocity.

The separation of the long-chain nucleic acid from the matrix is effected by rinsing the porous anion exchanger with a solution of high ionic strength (salt concentration).

In the purification of plasmide-DNA, for example from recombinant *E. coli* bacteria, various methods may be employed for the disintegration of the host cells. All of these methods after centrifugation at about 30 12,000 g produce the so-called clear lysate, a clear supernatant having been mostly rid of cell debris and chromosomal DNA, which supernatant contains plasmide-DNA, RNA, proteins and other soluble components. Here may be mentioned the lysozyme/Triton method or SDS method, respectively (cf. Maniatis et al.), the NaOH/SDS method (Birnboim, H.C. & Doly, I., 1979, Nucl. Acids Res. 7, 1513 to 1523; Ish-

35 Horowicz, D. & Burke, J.F., 1981, Nucl. Acids Res. 9, 2989 bis 2998), the phenol method (Klein, R.D. et al., 1980, Plasmid 3, 88 to 91) and the "Boiling" method (Holmes, D.S. & Quigley, M., 1981, Anal. Biochem. 114, 193 to 197).

The clear lysates, if they do not contain significant amounts ($\leq 0.01\%$) of ionic detergents such as SDS, 40 they may be directly purified by means of the method according to the present invention, whereby in response to the selection of suitable conditions of ionic strength (preferably from 0.5 to 0.7 M), for example, proteins, lipids, RNA and smaller molecules are separated via adsorption to the porous anion exchanger from long-chain DNA, more specifically DNA from plasmides, the latter materials being bound to the carrier material. The addition of urea to the loading buffer does not affect the binding behavior of the long-chain DNA, while, however, it optimizes the separation efficiency with respect to proteins. Thereby the high 45 capacity of this material of about 1 μg of nucleic acid per 1 mg of the porous matrix is specifically exploited for DNA in spite of the high molar excess of cellular RNA.

Unspecifically bound RNA and proteins are removed from the porous matrix in few washing steps by washing with buffer solutions of low ionic strength. Then the elution is carried out by extracting the anion exchanger with buffers of high ionic strength.

50 Due to the unusually high separation efficiency of the method according to the invention between RNA/protein, on the one hand, and long-chain DNA, on the other hand, subsequent RNase, and possible proteinase, treatment(s) as usually employed will not be required. If the clear lysate is SDS-free, such as after a potassium acetate precipitation or as produced by a lysozyme/TritonX-100, lysis, after adjustment of an ionic strength of from 0.5 to 0.7 M the lysate may be directly passed through the porous anion 55 exchanger in order to extract long-chain plasmides. Otherwise, SDS and proteins may first be extracted by phenolization and admixing with chloroform, followed by DNA extraction by means of the process according to the invention. Phenol dissolved in the lysate does not interfere with the plasmide-binding property of the porous anion exchanger.

If the volumes of the lysates are very large, it is recommended first to precipitate DNA with polyethylene glycol (PEG), ethanol or isopropanol. Then the pellet is dissolved in tris-buffer, the solution is adjusted to the desired ionic strength and passed through the porous anion exchanger. Thereby DNA is extracted from the solution, washed with buffers having lower ionic strengths in subsequent washing operations and thereafter re-extracted with tris-buffer of high ionic strength. Then, if desired, DNA may be desalted by a) dialysis, b) precipitation or c) gel permeation chromatography.

The plasmide-DNA isolated by means of the method according to the invention exhibits properties which are at least as good as those of the DNA isolated by using known purification methods. The plasmide DNA may be processed with restriction enzymes and DNA ligases; it is further capable of being sequenced or transfected.

λ -Phages are vehicles frequently used for the transportation of recombinant nucleic acids and are preferred over the plasmide vectors for many applications, as they

- a) after protein encapsulation (*in vitro* packaging) very efficiently introduce alien DNA into cells and, thus, are suitable for establishing comprehensive gene banks;
- b) may take in small as well as very large DNA fragments;
- c) have good storability;
- d) and are easy to cultivate.

Many cloning experiments start with establishing a λ -gene bank, and more specifically a randomly established gene bank. As in this stage only an insufficiently characterized DNA is employed, warranting biological safety is often a problem. Thus, for example, in cloning oncogenetic substances or viral sequences (HTLV-III/LAV-1) safety strains and safety phages of the biological safety level 2 (B2) have to be employed and processed under high laboratory safety conditions (L2 or L3; ZKBS, Berlin; cf. V-th revised version of handling newly re-combined DNA). In the course thereof, work-up steps such as centrifugations, and more particularly elaborate, time-consuming and expensive cesium chloride-gradient centrifugations and harvesting the phages constitute safety problems for laboratory and staff.

The method according to the inventions renders it possible to purify phages /phage-DNA by evading centrifugation steps. A grown or lysed bacterial culture may be completely worked up, if desired, in a sterile bank to yield λ -DNA having a purity conforming to that of cesium chloride-purified preparations.

Also a single-stranded DNA, for example M13 phage-DNA, can be purified by using the method according to the present invention. From cell lysates single-stranded DNA in high yield and purity may be used for sequencing and hybridization experiments. After the phages have been isolated, the single-stranded DNA is released and adsorbed on the porous anion exchanger. The interfering components are removed by washing.

For an isolation of cellular DNA from tissue of various origin the material is disintegrated and digested using known methods. Thus, a mechanical homogenization, for example under nitrogen, in a ball mill or by efficient maceration and shearing of the material, is followed by a proteolytic digestion in the presence of denaturing and/or solubilizing agents. Proteinase K is a preferred enzyme for the proteolytic digestion, as it efficiently leads to a lysis of cells and cell nuclei even in the presence of 1% of SDS and EDTA. According to prior art, SDS and the proteins have to be removed by time-consuming liquid-liquid extractions, which steps are followed by a dialysis and precipitation of DNA. This procedure is elaborate and difficult to automate. However, the method according to the invention allows to bring long-chain DNA into solution under mild conditions from the sample materials as mentioned above, to fix the DNA on the porous anion exchanger while evading any steps of phenol extraction, and subsequently to elute the DNA under mild conditions in a small volume (0.5 to 5 ml, and preferably 1 to 3 ml).

Infections with viruses play an important role in transfusion and transplantation medicine and generally in immunosuppressed patients. For example, an acute CMV (cytomegalovirus) infection can be detected by an analysis of renal excrements. According to the state of prior art the bacteria are separated from urine by a filtration step or low-speed centrifugation step, and thereafter the virus-DNA is released from the protein sheath and purified by concentration as simultaneously occurring. To this end ultracentrifuges were used in prior art.

The method according to the invention utilizes the described porous anion exchanger by lysing the CMV viruses *in situ* by addition of urea, detergent and buffer, whereupon the DNA (130 to 150 x 10⁶ Dalton) is released. The DNA is then concentrated by adsorption onto the porous anion exchanger and washed with buffer solutions having low ionic strength. Thereafter the DNA is eluted using a buffer of high ionic strength.

If further analysis is followed by a dot-blot technique, it is not required to desalt the DNA.

The use of a porous anion exchanger consisting of a chromatography material on the base of silicagel, diatomite, aluminum oxide, titanium dioxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the above-mentioned carrier

materials, the surfaces of which have been modified so that the matrix exhibits anion exchanger activities, warrants the advantages of the method according to the invention. The particle size of the porous matrix based on silicagel is, for example, 15 to 250 μm , and preferably 25 to 40 μm , and the pore diameter is 50 to 2,500 nm, and preferably about 400 nm.

5 The device for carrying out the method according to the invention consists of a container made of a material which is resistant to the operation conditions in accordance with the method of the invention. The container receives the porous anion exchanger and has at least one inlet and outlet openings each.

10 Figure 1 shows schematically the container, according to a preferred embodiment, for the porous anion exchanger consists of a cartridge 1, which preferably forms a substantially cylindrical hollow body and the side walls 2 of which consist of a material which is resistant to the working conditions (presence of more or less aggressive chemicals and corrosive salts). The side walls 2 preferably are made of a plastics material. Particularly simple is the preparation of the cartridges by using a shrink tube, for example one made of polytetrafluoroethylene (PTFE).

15 The inlet opening 3 and outlet opening 4 are delimited by filters 5 a and 5 b. In a preferred embodiment the filter consists of a hydrophilic material such as glass, hydrophilic plastics or plastics material coated with a hydrophilic material. However, hydrophobic materials may also be employed. The inlet opening 3 may optionally be shaped so that a Luer Lock system 6 is directly connectable to the inlet cannula.

20 The outlet opening 4, in a preferred embodiment, has an internal tube 8, preferably made of silicone, which is connected to the filter 5 b and preferably does not exceed the end of the outlet cannula 9. The eluate from the cartridge is discharged by a tube 10 which preferably is made of a plastics material, and particularly of a hydrophilic plastics material. Nevertheless, hydrophobic plastics materials such as PTFE may be used as well. The container may also be manufactured by injection molding.

25 Figure 2 shows schematically another preferred embodiment of the container according to the invention, which can preferably be produced by means of injection molding. The reference signs have the following meaning:

- 1 cartridge
- 2 wall
- 3 inlet
- 4 outlet
- 30 5a porous fritt
- 5b porous fritt
- 7 inlet tube
- 9 outlet tube
- 11 porous resin

35 Figure 3 shows still another preferred embodiment of the container according to the invention. Also this container can be produced by injection molding. The reference signs have the following meaning:

- 1 cartridge
- 2 wall
- 40 3 inlet
- 4 outlet
- 5a porous fritt
- 5b porous fritt
- 6 luer-lock connector
- 45 7 inlet tube
- 9 outlet tube
- 11 porous resin

The internal volume of the container for the porous matrix 11 depends on the intended use. Usually for analytical procedures the internal volume is about 0.02 to 5 cm^3 , and preferably 0.1 to 1 cm^3 . If solutions containing nucleic acids and other materials are to be purified on a preparative scale, containers having larger dimensions may be used as well. The porous resin 11 consists of a silicagel-based anion exchanger. The pore diameter of the material is 50 to 2,500 nm, and preferably about 400 nm, at a particle size of from 15 to 250 μm , and preferably 25 to 40 μm .

55 The mixture of disintegrated cells from tissue or body liquids, after proteolysis under mild conditions optionally in combination with the application of mechanical means, is introduced into the cartridge via the inlet opening and comes into intimate contact with the porous anion exchanger. Thereupon the anion exchanger extracts the long-chain nucleic acids from the mixture, whereas the other substance will leave the cartridge via the outlet opening. Attention is to be paid that the applied mixture of the digested material has

a low ionic strength. For example, at an ionic strength of about 300 mM of NaCl long-chain RNA and DNA are adsorbed, whereas proteins and lower molecular weight substances are not adsorbed to a significant extent; at concentrations higher than 500 mM of NaCl only long-chain single-stranded DNA and double-stranded DNA are bound, while at salt concentrations around 700 mM NaCl only long-chain double-stranded

5 DNA will be adsorbed on the porous anion exchanger.

Figure 4 demonstrates the typical elution profiles in NaCl-gradient elution at pH 7.0. The absorbance of different substances at 260 nm is plotted versus NaCl concentration. This exemplary diagramm shows the very good separation of the biomolecules. The dotted area symbolizes the range in which proteins, polysaccharides, low molecular weight metabolites and dyes are eluting off the anion exchanger. This happens in the range of from 0 to 0.4 M NaCl. At 0.1 M, for example, are eluting nucleotides, whereas the standard protein BSA (bovine serum albumin) elutes at 0.3 M NaCl concentration. The decamer linker, however, elutes at about 0.4 M NaCl. From the graph it can be taken that tRNA elutes at 0.5 M, 5 S RNA at 0.65 M, 16 S and 23 S rRNA and mRNA between 0.8 M and 0.9 M, M13-phage and other single-stranded (ss) DNA at 1.1 M, double-stranded (ds) DNA of 150 basepairs at slightly below 1.2 M and finally plasmid 10 DNA, for example such of the λ-phage, at 1.3 M NaCl, respectively, the latter one slightly overlapping with the former one. The values are determined only approximately because they might be varying dependent on the experimental conditions as the one skilled in the art expects.

If the porous anion exchanger is synthesized under conditions which do not result in a maximum surface charge density, then the separation profile is altogether shifted to lower ionic strengths, whereas the 20 separation efficiency is not significantly affected. This latter effect is even desired if the DNA must be eluted at a lower salt concentration.

After the sample has left the cartridge, the cartridge is carefully rinsed with a washing solution of the desired ionic strength (as set forth above), whereupon the long-chain nucleic acids are desorbed from the porous anion exchanger. This is effected by eluting with a solution of high ionic strength. To this end, in the 25 simplest case the second solution may be introduced through the same inlet opening 3 and be drained through the same outlet opening 4. However, there may also be used cartridges, if desired, which comprise different inlet openings and different outlet openings, respectively, for the solutions having low ionic strength and high ionic strength.

In a further embodiment the method according to the invention may be realized in practice as a "batch" 30 procedure which is distinguished by particularly simple handling. The batch procedure has the advantage of preventing shearing forces to high molecular weight nucleic acids. With this procedure, nucleic acids up to 500,000 basepairs (molecular weight approximately 300 million dalton) can be isolated on a preparative scale without degradation of the shearing force of sensitive molecules. A porous anion exchanger suitable for extracting long-chain nucleic acids is charged in a reaction vessel in a sufficient amount and intimately 35 mixed with the sample to be extracted, with the ionic strength of the solution being adjusted as indicated above. The long-chain nucleic acids are adsorbed on the porous anion exchanger. The contaminating components are removed by several washing steps. Thereafter the longchain nucleic acids are separated under mild conditions from the anion exchanger by elution using a buffer having the desired ionic strength. The porous anion exchanger is based on a surface-modified chromatography material from silicagel, 40 diatomite, aluminum oxide, titanium dioxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the above-mentioned carrier materials. Based on modified silicagel the pore diameter is 50 to 2,500 nm, and preferably about 400 nm, and the particle size of the is 15 to 250 μm, and preferably 25 to 40 μm.

The invention is further illustrated by means of the following examples:

45

EXAMPLE 1

The preparation of a plasmide (2860 base pairs) is carried out as follows:

Subsequently to the alkali/SDS digestion procedure a 100 ml culture is centrifuged in LB-ampicillin

50 medium (see Maniatis et al.) with plasmide-transformed HB-101 E. coli cells at 5000 g and 5 °C for 10 minutes. The supernatant is carefully decanted, and the cell pellet is resuspended in 2 ml of 50 mM glucose, 25 mM of Tris-HCl pH 8.0, 10 mM of EDTA.

The sample is allowed to sit at 20 °C for 5 minutes. Then 4 ml of a freshly prepared 1% SDS-solution in 0.2 M NaOH are added thereto and carefully admixed, and the mixture is incubated on ice for 5 minutes.

55 Thereafter, 3 ml of a cold sodium acetate solution (3M Na-acetate, 2M acetic acid) are added thereto and carefully admixed, and the mixture is incubated on ice for another hour. After 10 minutes of centrifugation at about 10000 g, 10 °C a clear plasmide-containing supernatant is obtained. If potassium acetate is used instead of sodium acetate, most of the SDS is precipitated.

In the case of large lysate volumes it is recommended first to precipitate the DNA with PEG, ethanol or isopropanol. Then the pellet is dissolved in 10 mM of Tris-buffer pH 7.5, 1 mM of EDTA, adjusted to 0.6 M of NaCl and passed over 200 mg of the anion exchanger. Thereby the DNA (about 100 µg) is extracted from the solution. In the subsequent washing step the gel phase is washed with 0.8 M NaCl, 50 mM of Tris-HCl buffer pH 7.5, 1 mM EDTA and extracted with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer pH 7.5, 1 mM EDTA. Thereafter the DNA may be desalted by dialysis, precipitation or gel permeation chromatography.

EXAMPLE 2

The preparation of λ-phage DNA is carried out as follows:
A grown and lysed λ-phage/E. coli culture (50 ml) is centrifuged at 5000 g and room temperature for 15 minutes or allowed to sit on ice for 30 minutes (cf. Maniatis, T. et al.). The supernatants or parts thereof are filtered through narrow-pore sterile filters, for example 0.45 µm, to retain intact cells or floating cell debris.

The suspension of phages is efficiently rid of cellular DNA by passing it through a cartridge (Fig. 1) at an ionic strength of from 0.5 to 0.7 M NaCl. The bed volume of the porous anion exchanger is to be selected so that the capacity is sufficient for the cellular DNA released from the lysed cells (about 200 mg of porous matrix per 100 ml of lysate).

The filtrate is treated with EDTA (200 mM). Upon simultaneous addition of 4 M of urea the DNA of the phages is released and by means of another filtration through the cartridge specifically adsorbed on the anion exchanger. Then the cartridge is washed with 0.8 M NaCl, 50 mM Tris-HCl buffer pH 7.5, 1 mM EDTA, and the DNA is eluted with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer pH 7.5, 1 mM EDTA.

The phage DNA thus obtained may be precipitated with PEG or isopropanol. It is also possible to desalt the phage DNA by means of a dialysis (cf. Maniatis, T. et al.). A DNA having high purity is obtained.

EXAMPLE 3

The preparation of M-13 phage DNA is carried out as follows (an analogous procedure is used for the preparation of single stranded DNA):

The phage lysate is obtained in a conventional manner (cf. EXAMPLE 2) by disintegration of the cells. After the removal of the cell debris, for example by centrifugation at 5000 rpm for a period of 5 minutes RNase A is added to a final concentration of 10 µg/ml, and an incubation is allowed to occur at 37 °C for 30 minutes. If the volume of the lysate is too large, a PEG (polyethylene glycol) precipitation of the phage is recommended. 0.3 volumes of 30% PEG and 1.5 M sodium chloride are added and well admixed, and the mixture is allowed to sit on ice for 30 minutes. The precipitated bacteriophage particles are separated from the solution by centrifugation at 10,000 g for 15 minutes. The supernatant is carefully aspirated, and the phage pellet is dissolved in 20 µl of 10 mM Tris, 1 mM EDTA, pH 7.5. Another part by volume of extraction buffer (2 % TritonX-100®, 7 M urea, 100 mM EDTA, pH 7.5) is added, and the mixture is heated at 50 °C for 15 minutes to release the single-stranded DNA.

The cartridge is equilibrated with a buffer of low ionic strength comprising 400 mM sodium chloride, 50 mM MOPS (3-N-morpholino-propanesulfonic acid), 15% ethanol and 1 mM EDTA at pH 7.0. The sample is passed through the cartridge. Then the cartridge is carefully washed with a buffer having a sodium chloride concentration of 750 mM and otherwise a composition as mentioned above. The single-stranded M-13 DNA may be eluted using an elution buffer having a composition of 1.1 M NaCl, 50 mM MOPS, 15% ethanol and 1 mM EDTA at pH 7.0.

EXAMPLE 4

The isolation of cellular DNA from sperm is carried out as follows:

One hundred µl of sperm are suspended in 1 ml of 500 mM NaCl, 10 mM EDTA, 40 mM DTE, 10 mM Tris-HCl buffer pH 7.5, 1% Triton, 4 M urea and 20 µg/ml of proteinase K and incubated at 37 °C for 2 hours. After centrifugation at about 5000 g for 5 minutes the supernatant is passed through the separating gel in a cartridge. The flow velocity of the supernatant through the cartridge is about 1 ml/min.

Alternatively the "batch" process may be used. In said process the supernatant is intimately mixed with the separating gel by rotation in a 1.5 ml Eppendorf reaction vessel for 15 to 30 minutes. The next step comprises washing the gel five times in the batch process or washing the gel in cartridge with 5 ml of washing buffer (800 mM NaCl, 50 mM Tris-HCl buffer pH 7.5, 1 mM EDTA), followed by the elution with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer pH 7.5, 1 mM EDTA. The elution yield is higher than

80%. The DNA may further be desalting by dialysis or precipitation (cf. EXAMPLE 1). The DNA is cuttable with restriction enzymes and is suitable for analysis with the Southern-Blot method (cf. T. Maniatis et al.).

EXAMPLE 5

5 The preparation of genomic DNA from liver biopsy material is carried out as follows:
 Liver biopsy material is mechanically homogenized according to the Potter procedure or any comparable method. To the homogenate proteinase K lysis buffer (cf. EXAMPLE 3), 10-fold volume, is added and the mixture is incubated at 37 °C for 2 hours. The following work-up steps are as described in
 10 EXAMPLE 4.

EXAMPLE 6

15 The preparation of papilloma-virus DNA from verruca biopsy tissue is carried out as follows:
 After a mechanical disintegration (liquid nitrogen, ball mill, mechanical squeezing) of verruca biopsy material, in the same manner as described in EXAMPLE 5 ten times the amount of lysis buffer is added, the mixture is incubated at 37 °C for 6 hours, and the DNA is worked up as described in EXAMPLE 4. The procedure provides a high molecular weight DNA, which is a mixture of cellular DNA of the human cells and papilloma-virus DNA from the proteolytical digested and lysed papilloma virions.
 20

EXAMPLE 7

25 The preparation of CMV (cytomegalovirus) DNA from urine is carried out as follows:
 CMV viruses are lysed *in situ* upon addition of 4 M urea, 1% Triton, 500 mM NaCl, 50 mM Tris-HCl buffer pH 7.5. The DNA (130 to 150 x 10⁶ Dalton) is released via adsorption on the porous matrix, concentrated in the cartridge shown in Figure 1 and washed as described in EXAMPLE 5. Then the DNA is eluted as described in EXAMPLE 4. Since these operations are usually followed by a Dot-Blot procedure which anyway requires high salt concentrations to be present for binding the DNA to a membrane (nitrocellulose, nylon), the eluted DNA solution is to be adjusted to concentrations of 0.1 M sodium hydroxide and about 2 M sodium chloride. Then a Dot-Blot is directly possible in the devices as conventionally used, for example Minifold I and II by Schleicher & Schüll, West Germany.

EXAMPLE 8

35 Removal of nucleic acids from protein solutions:
 To 5 ml of a BSA (bovine serum albumin) solution (1 mg/ml) there were added 50 ng of pBr 322 plasmid having tetracycline resistance (transformation equivalent about 800 colonies). The obtained solution was adjusted to 0.3 M NaCl to prevent the BSA from being bound to the chromatographic material, and then twice purified over a cartridge containing 250 mg of chromatography material (flow rate 5 ml/hour).
 40 Then the cartridge was washed with 1 M NaCl, 50 mM MOPS pH 7.0, and the bound DNA was eluted with 1.5 M NaCl, 15% ethanol, 1 mM EDTA and 50 mM MOPS pH 7.0, precipitated with isopropanol and transformed into E. coli. 800 colonies were counted. The effluent was dialyzed in a parallel operation, and then 100 µl were also transformed. No resistant colonies could be determined. A comparison of the transformation rates of the initial solution and of the eluate allows the conclusion to be drawn that
 45 approximately 100% of the DNA present had been removed by using the cartridge.

EXAMPLE 9

50 Removal of nucleic acids from therapeutic protein preparations:
 10 ml of human IgG (5 mg/ml) were traced with 500 pg Eco RI linearized pBR 322 plasmid DNA (10 pg DNA/mg protein). The linearized pBR 322 were labelled with ³²P to an activity of 5 x 10⁶ counts/min.
 The solution was adjusted to 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0, to prevent binding of the IgG to the chromatographic resin. The protein nucleic acid solution is pumped through a chromatography column (1 cm x 5 cm) filled with 2 g of the anion exchange resin at a flow rate of 1 ml/min. The flow through fraction was collected and the radioactivity was counted.
 The column was washed with 50 ml 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0, at a flow rate of 5 ml/min. The bound nucleic acid was eluted with 1.5 M NaCl, 25 mM Na-phosphate, pH 7. The eluate fraction was collected and precipitated with 1 vol. iso-propanol. The precipitated nucleic acid was dissolved

in 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0 and the radioactivity was counted.

Result:

5

Starting activity:	500,000 cpm
Flow through fraction:	15,000 cpm
Wash-fraction:	10,000 cpm
Eluate fraction:	475,000 cpm

10

A comparison of the radioactivity led to the conclusion that with this anion exchanger > 95% of the nucleic acids present in a therapeutic protein sample can be removed, reducing the nucleic acid content below 1 pg.

15

EXAMPLE 10

Isolation of nucleic acids from protein solutions for analysis of nucleic acid content:

Low nucleic acid contents (\leq 100 pg/ml) in concentrated protein solutions (> 2 mg/ml) cause problems

20 in quantitative analysis of the nucleic acid content of therapeutic protein preparations. For the sensitive analysis by the dot-hybridisation method the protein has to be removed from the nucleic acid. The classical method of proteinase K digestion, phenol/chloroform extraction lead to unrepeatable results and loss of nucleic acids and prevent the quantitative analysis.

Extraction with the silicagel based anion exchanger gives a reproducible result, with a recovery of the 25 isolated nucleic acid, without protein contamination, which is suitable for quantitative analysis.

10 mg of mouse IgG of unknown nucleic acid content were dissolved in 5 ml 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0. A cartridge (0.4 ml) filled with 200 mg silicagel based anion exchanger was equilibrated with 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0 and the IgG solution was forced through at a flow rate of 0.5 ml/min. The cartridge was washed with 10 ml 0.3 M NaCl, 0.025 M Na-phosphate, pH 7.0, at a flow rate 30 of 2 ml/min. The bound nucleic acid was eluted with 1.5 M NaCl, 0.025 M Na-phosphate, pH 7.0, and precipitated with 0.8 vol. i-prop. The nucleic acid was dissolved in 50 μ l 1 mM Tris-HCl, pH 7.0 and the analysis was done by a dot-hybridisation method with a specific mouse-cDNA clone. The efficient binding and quantitative recovery of nucleic acid at the above conditions permit the quantitative analysis of the nucleic acid content in protein solutions even at extremely low nucleic acid contents and very high protein 35 concentrations.

Claims

1. A method for the separation of long-chain nucleic acids from other substances from solutions containing nucleic acids and other materials avoiding the step of a chloroform and/or phenol extraction and/or density gradient centrifugation
40 wherein the long-chain nucleic acids are fixed on a porous anion exchanger the anion exchanger having a particle size of from 15 to 250 μ m and a pore diameter of 50 to 2500 nm,
whereas the substances to be separated therefrom are washed out from the anion exchanger using a washing solution having an ionic strength below the elution point of the long-chain nucleic acid to be 45 separated,
and the fixed long-chain nucleic acids are subsequently removed from the anion exchanger using a washing solution of high ionic strength.
2. The method according to claim 1, characterized in that the porous anion exchanger consists of a material for chromatography on the base of silicagel, diatomite, aluminum oxide, titanium oxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the above-mentioned carrier materials having been modified with respect to their surface.
50
3. The method according to claim 2, characterized in that the particle size of the silicagel-base material is from 15 to 250 μ m and the pore diameter is about 50 to 2500 nm.
55

4. The method according to anyone of claims 1 to 3 characterized in that the long-chain nucleic acids are separated in the presence of buffer solutions containing ethanol.
5. The method according to anyone of claims 1 to 4, characterized in that the materials employed have hydrophilic surfaces.
6. The method according to anyone of claims 1 to 5, characterized in that the disintegration is effected under mild conditions by means of an enzymatic proteolysis and/or in the presence of detergents and/or in the presence of denaturing agents or in combination with mechanical procedures.
- 10
7. The method according to anyone of claims 1 to 6, characterized in that the long-chain nucleic acids are separated from the protein in an amount of more than 99%.
- 15
8. The method of anyone of claims 1 to 7, characterized in that the separation of the long-chain nucleic acids is carried out in a batch process.
9. Use of an anion exchanger for the separation of long-chain nucleic acids in a method of anyone of claims 1 - 8.
- 20
10. Use according to claim 9, characterized in that the porous matrix consists of a material for chromatography on the base of silicagel, diatomite, aluminum oxide, titanium oxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the above-mentioned carrier materials having been modified with respect to its surface.
- 25
11. Use according to claims 9 or 10, characterized in that the particle size of the silicagel-base material is from 15 to 250 µm and the pore diameter is from 50 to 2,500 nm.
- 30
12. Use according to claim 11, characterized in that the particle size of the silicagel-base material is from 25 to 40 µm and the pore diameter is about 400 nm.
13. Use according to anyone of claims 9 to 12, characterized in that the long-chain nucleic acids are separated from the protein in an amount of more than 99%.
- 35
14. Use of a device for carrying out the method according to claims 1 to 8, consisting of a porous anion exchanger in a cartridge (1) having at least one inlet opening (3) and at least one outlet opening (4) and side walls (2) consisting of plastic material, the anion exchanger consisting of a material for chromatography on the base of silicagel, diatomite, aluminum oxide, titanium oxide, hydroxylapatite, dextran, agarose, acrylamide, polystyrene, polyvinyl alcohol or other organic polymers, derivatives or of copolymers of the abovementioned carrier materials having been modified with respect to its surface, said anion exchanger having a particle size of 15 to 250 µm and a pore diameter of 50 to 2,500 nm.
- 40
15. Use of a device according to claim 14, characterized in that the plastics material of the side wall (2) is PTFE.
- 45
16. Use of a device according to anyone of claims 14 or 15, characterized in that the side walls (2) of the cartridge (1) consist of a hydrophilic material.
17. Use of a device according to anyone of claims 14 to 16, characterized in that filters (5a) and (5b) defining the cartridge (1) consist of a hydrophilic material.

50 Patentansprüche

1. Verfahren zur Trennung von langkettenigen Nucleinsäuren von anderen Substanzen aus Lösungen, die Nucleinsäuren und andere Stoffe enthalten, unter Vermeidung des Schrittes einer Chloroform- und/oder Phenol-Extraktion und/oder einer Dichtegradientenzentrifugation, wobei die langkettenigen Nucleinsäuren an einem porösen Anionenaustauscher fixiert werden und der Anionenaustauscher eine Teilchengröße von 15 bis 250 µm und einen Porendurchmesser von 50 bis 2500 nm aufweist,

während die davon zu trennenden Substanzen aus dem Anionenaustauschermaterial ausgewaschen werden mittels einer Waschlösung mit einer Ionenstärke, die unterhalb des Elutionspunkts der abzutrennenden langkettigen Nucleinsäuren liegt,

und die fixierten Nucleinsäuren anschließend vom Anionenaustauscher mittels einer Waschlösung hoher Ionenstärke abgetrennt werden.

5 2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß der poröse Anionenaustauscher aus einem Material für die Chromatographie besteht auf der Grundlage von Kieselgel, Diatomeenerde, Aluminiumoxid, Titanoxid, Hydroxylapatit, Dextran, Agarose, Acrylamid, Polystyrol, Polyvinylalkohol oder anderen organischen Polymeren, Derivaten oder aus Copolymeren der genannten Trägermaterialien, die bezüglich ihrer Oberfläche modifiziert wurden.

10 3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß die Teilchengröße des Materials auf Kieselgel-Basis 15 bis 250 µm und der Porendurchmesser etwa 50 bis 2500 nm beträgt.

15 4. Verfahren nach irgendeinem der Ansprüche 1 bis 3, dadurch gekennzeichnet, daß die langkettigen Nucleinsäuren in Gegenwart von Ethanol enthaltenden Pufferlösungen abgetrennt werden.

20 5. Verfahren nach irgendeinem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß Materialien mit hydrophilen Oberflächen eingesetzt werden.

25 6. Verfahren nach irgendeinem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß der Aufschluß unter milden Bedingungen durch enzymatische Proteolyse und/oder in Gegenwart von Detergentien und/oder in Gegenwart denaturierender Agenzien oder in Kombination mit mechanischen Verfahren erfolgt.

30 7. Verfahren nach irgendeinem der Ansprüche 1 bis 6, dadurch gekennzeichnet, daß die langkettigen Nucleinsäuren in einer Menge von mehr als 99% vom Protein abgetrennt werden.

35 8. Verfahren nach irgendeinem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß die Trennung der langkettigen Nucleinsäuren im Batch-Verfahren erfolgt.

40 9. Verwendung eines Anionenaustauschers zur Abtrennung langkettiger Nucleinsäuren in einem Verfahren nach irgendeinem der Ansprüche 1 bis 8.

35 10. Verwendung Anspruch 9, dadurch gekennzeichnet, daß die poröse Matrix aus einem Material für die Chromatographie besteht auf der Grundlage von Kieselgel, Diatomeenerde, Aluminiumoxid, Titanoxid, Hydroxylapatit, Dextran, Agarose, Acrylamid, Polystyrol, Polyvinylalkohol oder anderen organischen Polymeren, Derivaten oder aus Copolymeren der genannten Trägermaterialien, die bezüglich ihrer Oberfläche modifiziert wurden.

40 11. Verwendung nach den Ansprüchen 9 oder 10, dadurch gekennzeichnet, daß die Teilchengröße des Materials auf Kieselgel-Basis 15 bis 250 µm und der Porendurchmesser etwa 50 bis 2500 nm beträgt.

45 12. Verwendung nach Anspruch 11, dadurch gekennzeichnet, daß die Teilchengröße des Materials auf Kieselgel-Basis 25 bis 40 µm und der Porendurchmesser etwa 400 nm beträgt.

50 13. Verwendung nach irgendeinem der Ansprüche 9 bis 12, dadurch gekennzeichnet, daß die langkettigen Nucleinsäuren in einer Menge von mehr als 99% vom Protein abgetrennt werden.

55 14. Vorrichtung zur Durchführung des Verfahrens nach den Ansprüchen 1 bis 8, bestehend aus einem porösen Anionenaustauscher in einer Kartusche (1) mit mindestens einer Einlaß- (3) und mindestens einer Auslaßöffnung (4) und Seitenwänden (2) bestehend aus Kunststoffmaterial, wobei der Anionenaustauscher aus einem Material für die Chromatographie besteht auf der Grundlage von Kieselgel, Diatomeenerde, Aluminiumoxid, Titanoxid, Hydroxylapatit, Dextran, Agarose, Acrylamid, Polystyrol, Polyvinylalkohol oder anderen organischen Polymeren, Derivaten oder aus Copolymeren der genannten Trägermaterialien, die bezüglich ihrer Oberfläche modifiziert wurden, wobei der Anionenaustauscher eine Teilchengröße von 15 bis 250 µm und einen Porendurchmesser von 50 bis 2500 nm aufweist.

15. Verwendung einer Vorrichtung nach Anspruch 14, dadurch gekennzeichnet, daß das Kunststoffmaterial der Seitenwand (2) PTFE ist.
16. Verwendung einer Vorrichtung nach irgendeinem der Ansprüche 14 oder 15, dadurch gekennzeichnet, daß die Seitenwände (2) der Kartusche (1) aus einem hydrophilen Material bestehen.
5
17. Verwendung einer Vorrichtung nach irgendeinem der Ansprüche 14 bis 16, dadurch gekennzeichnet, daß die die Kartusche (1) abschließenden Filter (5a) und (5b) aus einem hydrophilen Material bestehen.

10 **Revendications**

1. Procédé de séparation d'acides nucléiques à longue chaîne d'autres substances à partir de solutions contenant des acides nucléiques et d'autres matériaux, en évitant l'étape d'extraction au chloroforme et/ou au phénol et/ou l'étape de centrifugation par gradient de densité,
15 dans lequel les acides nucléiques à longue chaîne sont fixés sur un échangeur d'anions poreux, l'échangeur d'anions ayant une taille des particules comprise entre 15 et 250 µm et un diamètre des pores compris entre 50 et 2500 nm,
et dans lequel les substances à séparer sont séparées de l'échangeur d'anions par lavage à l'aide d'une solution dont la force ionique est en dessous du point d'élution de l'acide nucléique à longue chaîne à séparer,
20 et les acides nucléiques à longue chaîne fixés sont ensuite enlevés de l'échangeur d'anions à l'aide d'une solution de rinçage de force ionique élevée.
2. Procédé selon la revendication 1, caractérisé en ce que l'échangeur d'anions poreux est constitué d'un matériau pour chromatographie à base de gel de silice, de diatomite, d'oxyde d'aluminium, d'oxyde de titane, d'hydroxyapatite, de dextrane, d'agarose, d'acrylamide, de polystyrène, d'alcool polyvinyle ou d'autres polymères organiques, de dérivés ou de copolymères des matériaux supports sus-mentionnés ayant été modifiés eu égard à leur surface.
25
3. Procédé selon la revendication 2, caractérisé en ce que la taille des particules du matériau à base de gel de silice est comprise entre 15 et 250 mm et le diamètre des pores est environ compris entre 50 et 2500 nm.
30
4. Procédé selon l'une quelconque des revendications 1 à 3 caractérisé en ce que les acides nucléiques à longue chaîne sont séparés en présence de solutions tampons contenant de l'éthanol.
35
5. Procédé selon l'une quelconque des revendications 1 à 4, caractérisé en ce que les matériaux employés ont des surfaces hydrophiles.
6. Procédé selon l'une quelconque des revendications 1 à 5, caractérisé en ce que la désintégration est effectuée dans des conditions douces au moyen d'une protéolyse enzymatique et/ou en présence de détergents et/ou en présence d'agents dénaturants ou en combinaison avec des procédés mécaniques.
40
7. Procédé selon l'une quelconque des revendications 1 à 6, caractérisé en ce que les acides nucléiques à longue chaîne sont séparés des protéines dans une proportion supérieure à 99 %.
45
8. Procédé selon l'une quelconque des revendications 1 à 7, caractérisé en ce que la séparation des acides nucléiques est effectuée par un processus discontinu.
9. Utilisation d'un échangeur d'anions pour la séparation d'acides nucléiques à longues chaîne dans un procédé selon l'une quelconque des revendications 1 à 8.
50
10. Utilisation selon la revendication 9, caractérisée en ce que la matrice poreuse est constituée d'un matériau pour chromatographie à base de gel de silice, de diatomite, d'oxyde d'aluminium, d'oxyde de titane, d'hydroxyapatite, de dextrane, d'agarose, d'acrylamide, de polystyrène, d'alcool polyvinyle ou d'autres polymères organiques, de dérivés ou de copolymères des matériaux supports ayant été modifiés eu égard à leur surface.
55

11. Utilisation selon les revendications 9 ou 10, caractérisée en ce que la taille des particules du matériau à base de gel de silice est comprise entre 15 et 250 µm et le diamètre des pores est compris entre 50 et 2500 nm.

5 12. Utilisation selon la revendication 11, caractérisée en ce que la taille des particules du matériau à base de gel de silice est comprise entre 25 et 40 µm et le diamètre des pores est environ 400 nm.

13. Utilisation selon l'une quelconque des revendications 9 à 12, caractérisée en ce que les acides nucléiques à longue chaîne sont séparés des protéines dans une proportion supérieure à 99 %.

10 14. Utilisation d'un dispositif pour mettre en oeuvre le procédé selon les revendications 1 à 8, consistant en un échangeur d'anions dans une cartouche (1) possédant au moins une ouverture d'entrée (3) et au moins une ouverture de sortie (4) et des parois latérales (2) constituées de matière plastique, l'échangeur d'anions consistant en un matériau pour chromatographie à base de gel de silice, de diatomite, d'oxyde d'aluminium, d'oxyde de titane, d'hydroxyapatite, de dextrane, d'agarose, d'acrylamide, de polystyrène, d'alcool polyvinyle ou d'autres polymères organiques, de dérivés ou de copolymères des matériaux supports sus-mentionnés ayant été modifiés eu égard à leur surface, ledit échangeur d'anions ayant une taille de particules comprise entre 15 et 250 µm et un diamètre des pores entre 50 et 2500 nm.

20 15. Utilisation d'un dispositif selon la revendication 14, caractérisé en ce que la matière plastique des parois latérales (2) est du PTFE.

25 16. Utilisation d'un dispositif selon l'une quelconque des revendications 14 ou 15, caractérisé en ce que les parois latérales (2) de la cartouche (1) sont constituées d'un matériau hydrophile.

17. Utilisation d'un dispositif selon l'une quelconque des revendications 14 à 16, caractérisé en ce que les filtres (5a) et (5b) délimitant la cartouche (1) sont constitués d'un matériau hydrophile.

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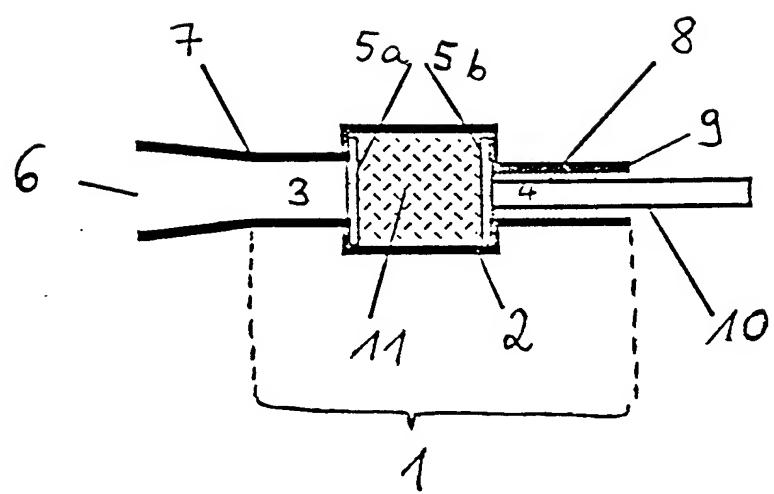


Fig. 1

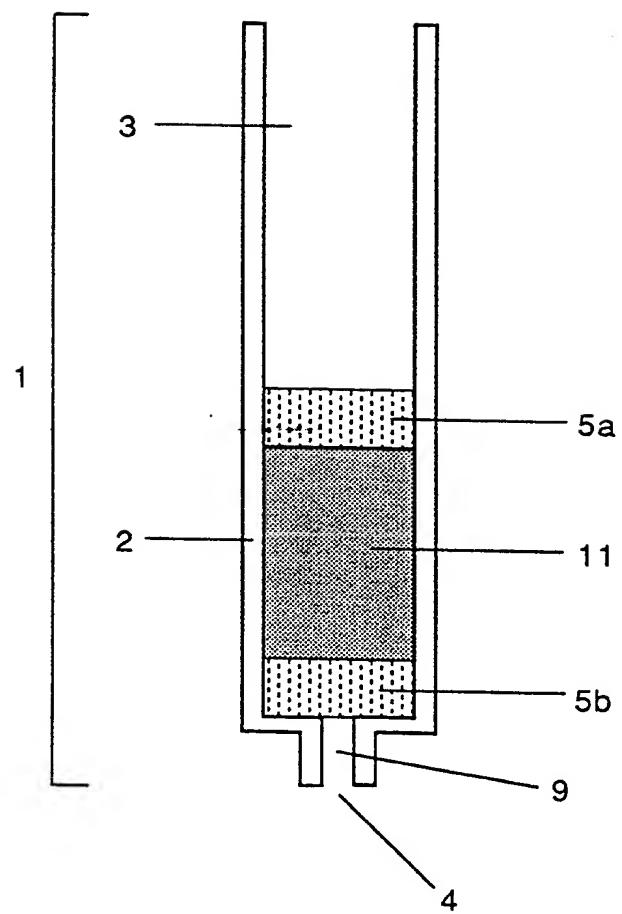


Fig. 2

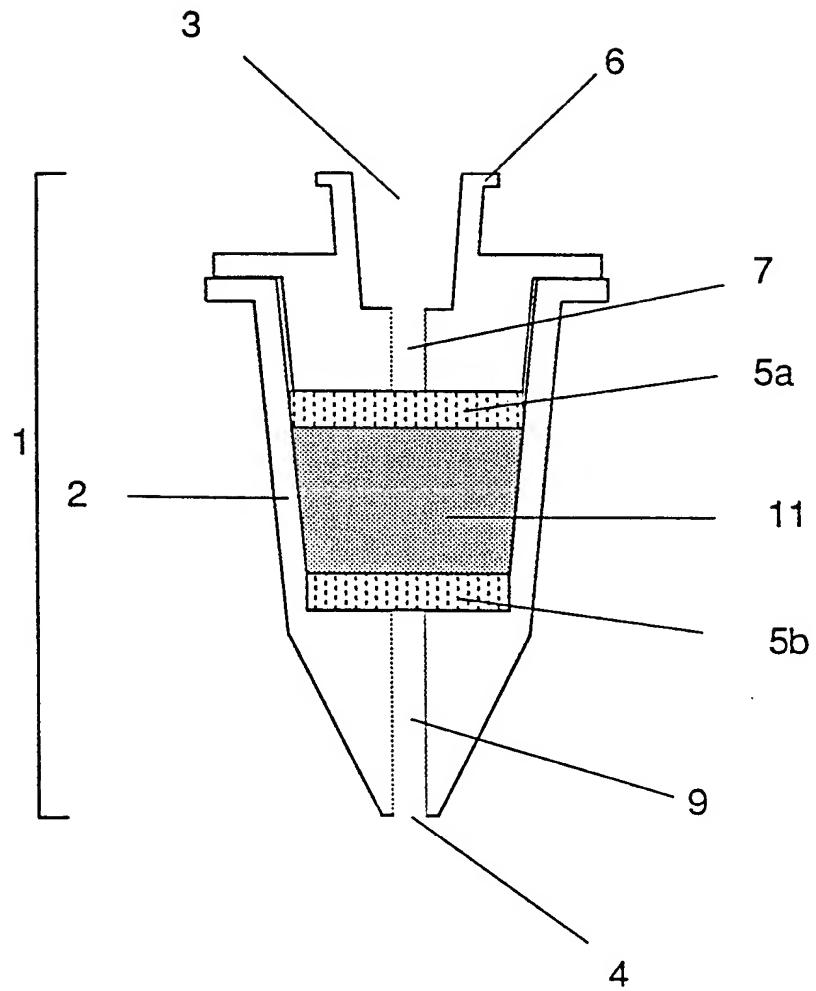


Fig. 3

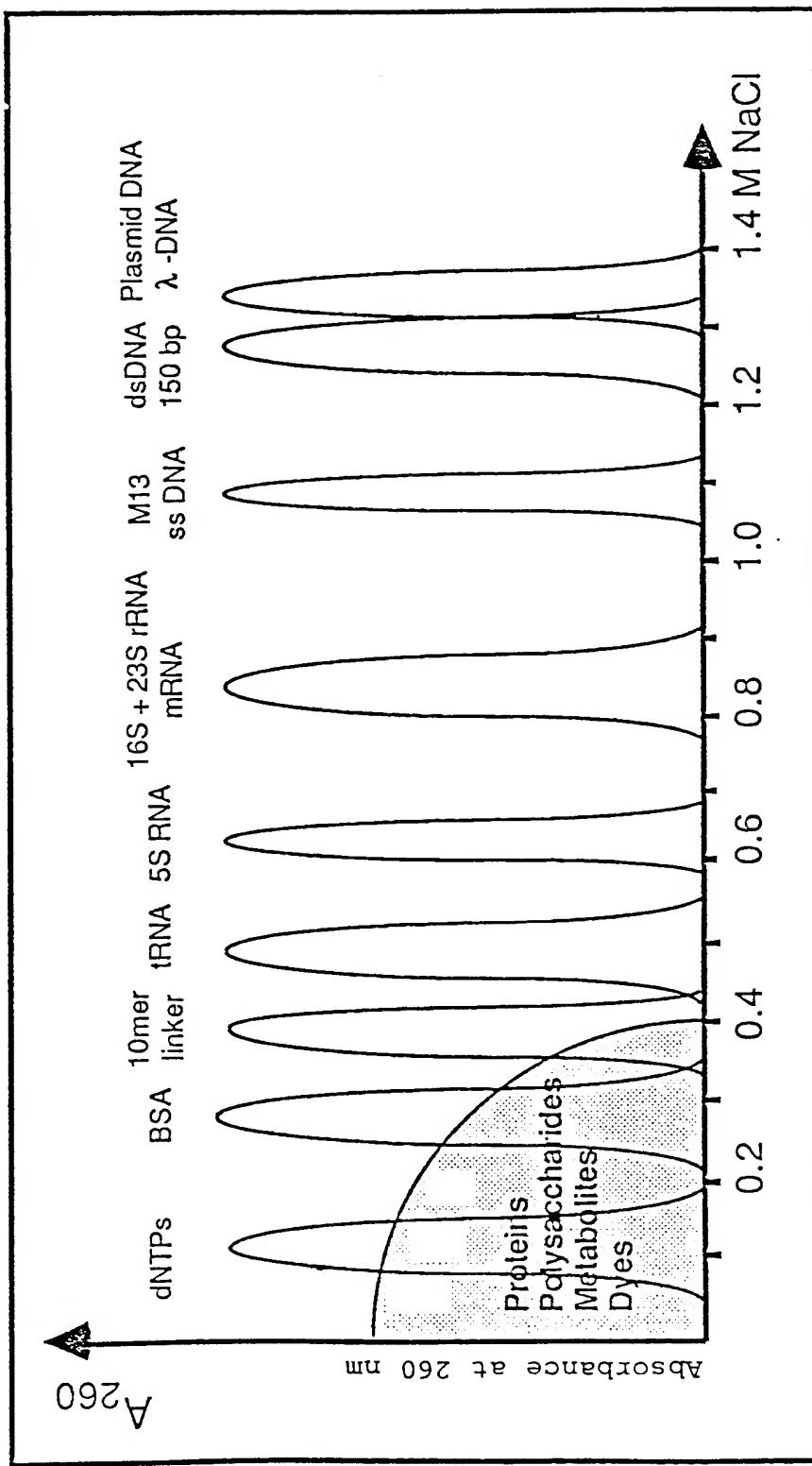


Fig. 4 Elution profiles in NaCl-gradient elution at pH 7.0.